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Studies on the Effect of Quinone Oxime Benzoyl Hydrazone* against Seedling Diseases of Various Cultivated Plants

by Dr. P. E. Frohberger

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Introduction

Seed treatment has given effective control of numerous plant diseases for very many years past. The most effective dressings have proved to be those which contain a low percentage of an organic mercurial compound as their active substance. The conventional mercurial compounds possess an all-round fungicidal efficacy. When applied as dressings, they act against all pathogens adhering to the outer surface of the seed. They disinfect the seed surface and prevent the development of seed-borne seedling infections without hindering seed germination or the growth of the plants. But in cases where the pathogen has already entered the seed, these dressings are ineffective. This means that such fungi as *Ustilago nuda* (Jens.) Kost. and *Ustilago tritici* (Pers). Jens. cannot be controlled by treating the seed with mercurial compounds, and moreover the results given by seed treatments against *Colletotrichum lindemuthianum* Sacc. and May. are often unsatisfactory. Furthermore, mercury salts are readily absorbed by the soil so that their fungicidal action is restricted to the immediate vicinity of the treated seeds. We were able to prove by employing the method described by Zoog (1), that the distance effect produced by commercial type mercurial preparations does not spread in the soil any more than 1 to 2 mm at the most from the surface of the seed. Consequently the effective range of the dressing is extremely small, which means that the growing seedling is afforded very brief and inadequate protection against

* Active substance of the two seed dressings, "Cerenox" and "Cerenox Special".

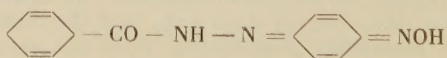
soil-borne infections. Riehm (2), for example, has expressed the opinion that beet seed treatment is a futile practice considering that effective control is given only against *Phoma betae* Frank adhering to the seed whereas the soil fungi, *Pythium debaryanum* Hesse and *Aphanomyces laevis* de By. are not controlled at all. The ratio for the frequency at which *Phoma*, *Pythium* and *Aphanomyces* occur as the pathogen of root rot is estimated at 7:4:2. This means that the probability of infection from the seed and from the soil is approximately the same (Braun, 3). A similar situation is encountered with numerous other cultivated plants such as peas, beans, flax, maize and cotton which are also frequently infected on a large scale by seedling diseases. Seed treatment of these crops, too, often proves to be unsatisfactory because it hardly has any effect against infections originating from the soil for the reasons given above. Considerable economic importance is attached to these seedling diseases because they are able to inflict enormous damage which either leads to large yield decreases or renders it necessary for farmers to re-cultivate the fields. Consequently there is a genuine demand for preparations which in their efficacy are superior to organic mercurial compounds for the purpose under discussion but which do not incur such high costs as a thermal or chemical soil disinfection.

Recently several organic compounds have gained practical significance as active substances for seed dressings. Although they do not possess the all-round efficacy of mercurial compounds, they are more effective against certain seedling diseases. The best known of these compounds are tetramethyl thiuram disulphide (TMTD) which is contained to 50—80 % in several seed dressings available on the world market; N-trichloromethyl mercapto-4-cyclohexane-1,2-dicarboximide ("Captan") contained to 75 % in "Orthocide 75 Seed Protectant"; 2,3-dichloro-1,4-naphthoquinone (Dichlone) of which "Phygon" has a 50 % content; tetrachloroparabenzquinone (Chloranil) which is marketed as a 96 % seed dressing formulation under the trade-name of "Spargon". Last year, Farbenfabriken Bayer Aktiengesellschaft, Leverkusen, introduced a special dressing for application against damping-off, marketed under the trade-name of "Cerenox". It contains 10 % of a new type of active substance, quinone oxime benzoyl hydrazone (COBH). It is not only effective against seedling diseases transmitted by the seed but also gives excellent control against soil-borne infections.

Description of the preparation

Quinone oxime benzoyl hydrazone (COBH) was submitted to us by Ewald Urbschat (4), to be tested for its suitability as a plant protection product. The usual tests *in vitro* produced negative results. Applied as a seed dressing in greenhouse sowing tests, it produced effects of a most interesting nature so that we decided to carry out more exacting tests.

The structural formula of COBH is:



It has a molecular weight of 241. In production it is yielded as a yellowish, odourless and non-irritant powder. Thermal decomposition occurs at approximately 195 °C. It is only very slightly soluble in water (5 mg/l), but readily soluble in soda solution or diluted caustic soda solutions, accompanied by a

change in colour to a deep reddish-brown. In several organic solvents it is moderately soluble, and readily soluble in dimethyl formamide. COBH is not volatile. Its vapour pressure is extremely low. No measured values were obtained up to 80° C. Accordingly, the maximum vapour pressure at 80° C is estimated to be 10⁻⁵ mm Hg.

In our studies, the preparation was applied chiefly as a dry dressing in the form of a fine-grained, highly adhesive powder, extended with talc. In certain instances it was also used undiluted or in alkaline solution as a wet dressing, or suspended in water as a slurry dressing. The method of application has no influence upon the effect. Its fungistatic or fungicidal efficacy *in vitro* is very slight in direct action on the fungus, and has no practical significance. It was not until relatively high concentrations were used, or at high rates of application, that the preparation produced a definite effect in the spore germination test against *Phytophthora infestans* (Mont) de By. and *Venturia inaequalis* (Cook) Aderh., and in the agar plate test against the mycelium growth of *Coniophora cerebella* Schroet., *Rhizoctonia solani* K., *Pythium debaryanum* Hesse, *Phoma betae* Frank and various mould fungi. The weak effect against the mycelium growth of *Phoma* and *Pythium* on agar nutrient media was not increased even when the preparation — applied for example to beet seed — was pressed into the nutrient substrate of the fungus. It is practically ineffective *in vivo*, too, when applied as a dry seed dressing against *Ustilago avenae* (Pers.) Jens., *U. nuda* (Jens.) Kost., *U. tritici* (Pers.) Jens., *Helminthosporium gramineum* Rabh. and *Fusarium nivale* (Ces.) Sor. The effect against *Tilletia tritici* (Bjerk.) Winter is quite considerable, but nevertheless inadequate for practical application. On the other hand, COBH is extremely effective as a dressing applied against seedling diseases of different seeds.

Dressing Tests

Sugar and fodder beets

The sowing tests were conducted in the greenhouse with standard sugar beet seed which was contaminated with *Phoma betae*. In two replications, batches of 50 beet seeds were set at a depth of 3 cm in wooden boxes measuring 25 × 25 × 10 cm. Ordinary compost was used for the seed bed. The compost was made up from various kinds of vegetable refuse supplied by market gardens, and was infested with numerous phytopathogenic fungi. From several breeds of seed, certain varieties were selected which had a high plant loss due to root rot, and which would contribute to intensifying the conditions for the dressing test because of their degree of infection and their susceptibility. The greatest incidence of root rot was always in the autumn, winter and spring months at temperatures between 12 and 15° C, on short days and in poor light conditions. Therefore, every attempt was made to retain these more or less natural conditions which are stimulating to the fungi and which prevent the seedlings growing out of the susceptible stage too rapidly. The seed was sown at regular intervals so that when the plants emerged, we were able to classify with adequate reliability the plants of the different beet seeds, and to carry out the counts separately according to the points of emergences and plants. In order to record delay and acceleration of germination, the count was made three times at intervals of two days after the plants started to emerge. During the remaining period of the tests, plants dying of fungus

attack were removed and recorded daily. After three to five weeks had elapsed, the plants had fully developed their first foliage leaves and had now reached a stage where they were very resistant to root rot, so that during this period it was possible to carry out the final evaluation of the test. This entailed determining the number of healthy emergence points which still had at least one healthy plant, and the total number of healthy plants per box as the decisive criterion for assessing the efficacy of the dressing. Furthermore, the number of all emerged plants was counted plus the number of plants which became seriously infected later or which perished.

A large number of comparison tests were carried out with COBH in this manner, which to a certain extent varied due to influences that were not covered experimentally, but which in practically all cases produced similar results. Therefore, by quoting the results of only a few tests (Tables 1, 2 and 3), a general picture will be given of what happened in practically all the tests.

The very first count made on the 14th day after the seed had been sown gave an indication that the seed treatment was a success. However, there was still no apparent difference between phenyl mercury acetate and quinone oxime benzoyl hydrazone. This would mean that the earliest infections caused at the beginning of germination were still being reduced by PMA to the same extent as by COBH. But when the second count was made, the situation had changed in favour of COBH which evidently eliminates later infections to a much greater degree than PMA. From the 16th to the 18th day, no more plants emerged from the untreated seed, and a large percentage of those already emerged died of root rot. The application of PMA and COBH led to a further increase in the numbers of emerged plants. This shows that those seedlings which were late in emerging had the advantage of a protective effect in the initial stage of their development, which must be attributed to the corresponding persistent efficacy of both dressings on the surface of the seed. During the further course of the test, very many old plants died of root rot. These had grown either from untreated seed or from PMA-dressed seed. The COBH treatment, on the other hand, afforded long-lasting protection. In

Table 1: Polygerm sugar beet seed in ordinary, infested compost.

Dry dressing	Conc. of active substance expressed in %	Rate of application expressed in g/kg	14th day		16th day		18th day		26th day			
			A	B	A	B	A	B	A	B	C	D
untreated	—	—	34	58	46	76	32	48	20	20	26	56
PMA	2.2 Hg	6	42	68	56	110	62	116	40	60	24	62
COBH	2.5	6	40	74	66	150	68	154	74	160	0	10
„	5.0	6	42	70	64	152	70	146	70	142	0	16
„	10.0	6	46	78	78	164	80	166	80	172	0	0

A = No. of healthy emergence points based on 100 beet seeds.

B = No. of healthy plants based on 100 beet seeds.

C = No. of dead emergence points based on 100 beet seeds.

D = No. of dead plants based on 100 beet seeds.

view of the fact that at this stage of the test, PMA no longer had any effect, it may be concluded that the plants were attacked by soil infections which occurred outside the very restricted effective zone of the PMA dressing. On the other hand, the persistent effect of COBH against these soil infections cannot be attributed to a larger effective zone of the preparation because its low volatility and its slight solubility in water make it appear extremely unlikely that there is a greater spread of effective concentrations in the soil. Our assumption that COBH is absorbed by the swelling seeds giving the seedlings interior protection over a certain period of time against infections developing in the soil even outside the effective zone of the dressing was strengthened by the results of later tests.

Table 2 gives the final results of five more beet tests (I to V) carried out under similar conditions. These tests show the effect of COBH in the concentration range of 0.01 to 100 %.

Table 2: Polygerm sugar beet seed in ordinary, infested compost. Evaluation four weeks after sowing.

Dry dressing	Conc. of active substance expressed in %	Rate of application expressed in g/kg	Healthy emergence points (A) and healthy plants (B) expressed in %									
			I		II		III		IV		V	
			A	B	A	B	A	B	A	B	A	B
untreated	—	—	34	60	18	33	50	75	47	83	26	47
PMA	2.2 Hg	6	56	115	37	52	57	96	66	136	52	111
COBH	0.01	6	—	—	—	—	—	—	49	85	—	—
„	0.1	6	59	111	—	—	—	—	50	122	—	—
„	1.0	6	82	168	40	76	54	98	59	142	70	162
„	2.5	6	—	—	40	62	76	172	—	—	60	137
„	5	6	77	165	62	124	82	168	65	149	77	181
„	10	3	74	151	—	—	86	170	68	155	75	179
„	10	6	72	163	—	—	—	—	—	—	—	—
„	15	6	—	—	66	150	84	190	76	178	61	143
„	20	6	72	166	—	—	—	—	—	—	—	—
„	50	6	62	135	—	—	—	—	62	132	73	170
„	100	6	75	155	—	—	—	—	—	—	—	—

The extent to which the results vary is most considerable in certain cases. This, however, is not surprising in view of the very many factors directly responsible for the occurrence of seedling diseases, and will simply have to be taken into account.

The differences between the efficacy of both preparations are very evident. COBH can produce a definite protective effect even at a concentration of only 0.1 %. Applied as a 1 % dressing, it equals or in fact surpasses the effect of

Table 3: Different seed specimens in ordinary, infested compost.

Seed variety	No. of healthy plants emerged from 50 (a and b) and 100 (c) beet seeds after 31 days					
	untreated		2.2 % Hg as PMA 6 g/kg		10 % COBH 3 g/kg	
	a and b	c	a and b	c	a and b	c
Sugar beets, variety unknown	12		23		45	
do.		20		49		106
do.	8		26		61	
Svalöfs Barres	5		41		27	
do.		9		71		71
do.	4		30		44	
Orig. Halbzucker CB	13		7		43	
do.		18		12		80
do.	5		5		37	
Dt. Barres	8		25		42	
do.		17		69		87
do.	9		44		45	
Olivfarb. od. gelbe Flaschen	14		28		26	
do.		18		39		67
do.	4		11		41	
Orig. Grünkopf CB	0		27		45	
do.		2		55		86
do.	2		28		41	
Weibulls rote Eckendorfer St. 11	1		10		31	
do.		2		39		74
do.	1		29		43	
Svalöfs Rubra II	33		20		43	
do.		55		52		61
do.	22		32		18	
Altenburger Tonnen	4		47		35	
do.		15		78		94
do.	11		31		59	
Babolnai sarga henger	4		30		15	
do.		10		61		32
do.	6		31		17	
Criewener gelbe	6		35		29	
do.		13		46		58
do.	7		11		29	
Productiva	58		35		38	
do.		73		77		92
do.	15		42		54	
Remlinger	4		29		50	
do.		4		64		74
do.	0		35		24	
Dickwanst	32		75		49	
do.		55		100		82
do.	23		25		33	

2.2 % Hg = 3.7 % PMA. By raising the concentration of active substance to 5 % or to 10 %, with a reduction of the rate of application to 3 g per kg of seed, a further increase in the effect can be attained under these difficult test conditions. Further increases of concentration, however, do not have any really positive effects, and even when COBH is applied undiluted there is no evidence of phytotoxic properties. Accordingly, the effective potency and the chemotherapeutic index appear to be extremely favourable.

Table 3 gives the final results of a beet test which was conducted with different seed specimens. This test clearly shows how much the incidence of root rot and the effect of dressing can be influenced by the variety and origin of the seed when the other conditions are alike. However, the question must remain unanswered whether it was the genetic or the anamnestic factors of the seed which were influential in this particular test which was carried out in July. In the summer season with its high temperatures and strong light conditions, infestations of root rot are generally not serious enough to allow significant effects of seed dressing to be recognized, so that after the seed had been sown the wooden boxes were placed for 10 days in an air-conditioned chamber at a temperature of 8 °C and a relative humidity of 95 %. As a result, germination was delayed and the susceptible stage in the development of the seedlings was prolonged. From the 11th day onwards, the boxes were exposed to the summer conditions of the greenhouse. Emergence started on the 12th day and was practically completed by the 15th day. The plants developed very rapidly. The test was evaluated on the 31st day. The arrangement of the test was the same as that described earlier.

Peas

The pea tests were arranged in a similar manner to the beet tests. 50 peas per box, at a depth of 3 cm in natural, strongly infested compost, with two replications. Marrow peas were chiefly used as seed in the very many tests conducted. These peas suffered the heaviest losses due to rotting of the seedlings in the soil, and therefore made it possible to give the dressings an extremely severe test. The pea tests were far less dependent upon low temperatures than the beet tests. Even at temperatures of up to 20 °C, and sometimes higher, the emergence of the untreated seed in the strongly infested soil remained very slight, so that the test conditions could still be regarded as

Table 4: Marrow peas, Alderman E 53, in ordinary, infested compost.
Uniform rate of application: 2 g dressing per kg of seed.

Dry dressing	Conc. of active substance expressed in %	10th day	12th day	14th day	24th day	
		A	A	A	A	B
untreated	—	12	19	20	9	14
PMA	2.2 Hg	53	61	61	39	22
COBH	5	72	80	83	78	7
„	10	70	78	78	79	2
„	15	75	85	87	79	8

A = No. of healthy plants which emerged from 100 seeds.

B = No. of diseased and dead plants which emerged from 100 seeds.

severe despite the high temperatures. The evaluation began with the count of the plants. It was made three times at intervals of two days, following emergence. During the further course of the test, a record was kept of the numbers of dying plants. In the final evaluation which was carried out after three to four weeks, the plants were dug out singly in order to record the numbers of diseased plants, too. It appeared justified to stop the test at the end of this period because as a rule no more diseases occur at this stage. The number of healthy plants which had developed from 100 seeds by the end of the test, was taken as the final result. Table 4 provides a general picture of the outcome of the test.

From 77 % of the untreated peas, no seedlings developed which were able to penetrate the 3 cm thick layer of soil. Apparently they were infested very early, and rotted in the soil. The majority of the emerged plants showed serious symptoms of disease in the period between the 14th and 24th day of the test. We attribute these symptoms to a late soil infection. Only 9 plants remained healthy enough for them to be expected to continue normal development. By dressing the seed with PMA, the earlier infection of the germinating seeds was apparently eliminated to a great extent so that out of 100 seeds, 61 seedlings reached the surface of the soil but later 22 of these, a relatively large number, became seriously infested or died due to subsequent infections which developed in the soil. On the other hand, the application of COBH considerably improved both the rate of emergence and the final result. From this it may be concluded that COBH affords the seedling protection not only at the beginning of its development when it breaks through the seed coat but also several days later when it has already grown to a point outside the dressing's effective zone. The protection given to young seedlings by the COBH

Table 5: Marrow peas, Alderman E 53, in ordinary, infested compost.
Uniform rate of application: 2 g dressing per kg of seed.
Evaluation 3 to 4 weeks after sowing.

Dry dressing	Conc. of active substance ex- pressed in %	No. of healthy plants emerged from 100 seeds									
		I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
untreated	—	1	2	4	1	12	1	3	8	1	5
PMA	2.2 Hg	45	46	51	32	48	51	15	41	38	44
COBH	0.01	2	—	2	—	—	—	—	—	—	—
"	0.05	—	—	29	—	—	—	—	—	—	—
"	0.1	23	—	45	35	38	39	54	—	—	49
"	0.5	—	—	64	68	—	—	73	—	68	70
"	1.0	90	67	74	66	50	62	65	84	—	69
"	2.5	—	85	—	82	—	—	83	—	66	87
"	5	—	92	88	72	—	—	77	—	72	73
"	10	91	84	82	81	82	83	75	84	74	78
"	15	—	89	—	84	—	—	—	—	—	—
"	20	—	—	—	—	—	—	—	—	—	75
"	30	85	—	—	81	—	—	—	—	—	77
"	50	84	66	—	66	—	—	—	64	68	65
"	100	81	—	—	—	85	59	—	—	—	—

dressing against soil infections, lasts almost throughout the susceptible stage in the development of the pea. Of the 84 plants which emerged on the average from 100 seeds treated with COBH, only 6 became diseased later, so that 78 healthy plants were obtained as the final result. A large number of identical tests were conducted, and they all produced similar results. Table 5 gives the final results of 10 tests, providing a general picture of the entire series.

Applied in the normal manner as a dry dressing, COBH produced at a concentration of only 0.1 % a most positive effect almost equal to that of PMA. When the content of active substance in the dressing is raised to 2.5 %, a considerable increase in effect is obtained, clearly demonstrating the greater efficacy of COBH as compared with PMA at the same concentration. Further increases in concentration only improve the effect slightly. Negative influences on the growth of the plants were not observed, and in fact growth was often stimulated. It was not until undiluted active substance, or active substance extended to 50 %, was applied that there was a certain decline in several cases, although we were not able to attribute this to a phytotoxic tendency of the preparation. Table 6 gives the average results of 9 tests in which the 100 % active substance produced poorer results than the 10 % dry dressing; the figures quoted are the numbers of healthy plants, and constitute most interesting data.

Table 6:

Dressing	First Count	Final Result
10 % COBH	76	80
100 % COBH	68	59
Difference	8	21

The difference in the number of plants which had penetrated the soil surface and made a healthy impression at the first count, might have suggested that the overdosage had caused a delay in germination or an inhibition of growth, had this difference not increased due to a high rate of infestation during the course of the test, and had there been any differences in the habitus of the healthy plants. However, it appears more probable that the strong overdosage of COBH caused an unusual weakening of the protection afforded against infection. The reason for this must be sought in the special mechanism of action of the preparation which after all only becomes effective with the cooperation of the plant.

Table 7 gives the final results of a pea test carried out with eight different seed specimens. These results illustrate the extent to which the success of a treatment depends upon the seed. Six different varieties of marrow peas were tested. With respect to these, it cannot be said to what degree the differences which arose constitute peculiarities of the varieties, or to what extent these differences must be attributed to environmental influences exerted during the development and the storage of the seeds. Comparison tests were made on three deliveries of the Alderman variety, two of which originated from the same harvest year (1953). They provide an example of the bearing which the anamnesis of the seed has upon the possible success of the seed treatment, irrespective of the variety. The test was conducted in the same manner as that already described.

Table 7: Different seed specimens of marrow peas (market produce) in ordinary, infested compost.

Uniform rate of application: 2 g of dressing per kg of seed.

Seed variety	No. of healthy plants emerged from 50 (a and b) and 100 (c) seeds after 3 weeks					
	untreated		2.2 % Hg as PMA		10 % COBH	
	a and b	c	a and b	c	a and b	c
Alderman E 1953	12		16		33	
1st delivery		22		34		63
	10		18		30	
Alderman E 1953	1		28		31	
2nd delivery		1		57		56
	0		29		25	
Alderman E 1954	20		25		42	
		34		54		81
	14		29		39	
Onward E 1954	12		23		47	
		20		54		88
	8		31		41	
Senator E 1954	3		2		25	
		9		3		43
	6		1		18	
Van Waverens Juwel, Hochzucht E 1954	17		30		38	
		32		56		76
	15		26		38	
Lincoln E 1954	19		35		50	
		41		75		98
	22		40		48	
Wunder von Kelvedon E 1954	6		12		31	
		13		22		60
	7		10		29	

Beans

The dressing tests with beans were conducted in the same manner as those carried out with peas. Strongly infested compost, and germination delayed by relatively low temperatures constituted severe testing conditions. The tests with peas and beans produced similar results.

Table 8 gives several final results as an example of the general outcome of the tests.

The bean tests carried out in infested soil also illustrate the general superiority of COBH over PMA. It is only when the seed is seriously infested by *Colletotrichum lindemuthianum* Sacc. and May, occurring as a seedling disease,

Table 8: Different seed specimens of beans (market produce) in ordinary, infested compost.

Uniform rate of application: 2 g of dressing per kg of seed.

Seed variety	Harvest year	No. of healthy plants emerged from 100 seeds after 3 weeks		
		untreated	2.2 % Hg as PMA	10 % COBH
Dwarf beans:				
St. Andreas	1954	24	76	68
Wachs Rheinland	1954	3	35	52
Hundert für eine	1954	21	31	51
Wachs Bitterhoffs Füllhorn	1954	32	71	80
Zucker Perl Perfektion	1954	33	48	68
Wachs Beste von allen	1954	10	59	98
Saxa o. F.	1954	41	90	85
Konserva, weißgrundig	1954	15	47	82
Dickfl. Zucker Brech	1954	23	86	98
Kaiser-Wilh.-Riesen	1954	18	80	90
Kaiser-Wilh.-Riesen	1952	50	80	94
Dopp. holl. Prinzeß	1953	49	64	84
unknown	1954	17	20	44
Runner beans:				
Ruhm v. Vorgebirge	1952	3	81	92

that the picture changes. Table 9 gives several results obtained in these tests. Beans A without visible spots, and beans B heavily covered with spots, were selected from the same batch of seed. C designates another batch which did not actually contain any spotted seed but which originated from heavily spotted pods.

Organic mercurial compounds are more effective than COBH against anthracnose of beans occurring as a seedling disease, especially when the seed is already suffering from advanced symptoms of the disease. A combination of both types of active substance appears to give the most effective control

Table 9: Beans in ordinary, infested compost.

No. of healthy plants emerged from 100 seeds 4 weeks after sowing.

Bean seed	untreated —	2.2 % Hg as PMA 2 g/kg	10 % COHB 2 g/kg	10 % COBH
				+ 3 % Hg as PMCl * 2 g/kg
A No spots	56	80	86	—
B Spotted	0	72	54	—
C No spots	58	95	79	95

* This preparation, which contains 10 % COBH and 3 % Hg as phenyl mercury chloride, is marketed under the trade-name of "Cerenox Special".

in this case. A similar situation is encountered in the control of leaf spot of beets (*Cercospora beticola* Sacc.), that is when the disease is transmitted by the seed. Here, too, a combination of both active substances has proved to be most effective.

Miscellaneous seed

Beets and leguminous plants are not the only species which suffer from seedling diseases developing in the soil. In fact most seeds are infested. Provided the pathogens were similar, it was presumed that here, too, COBH would produce its specific protective effect under suitable conditions. To strengthen this assumption, a number of exploratory tests were conducted using several batches of different types of seed infested with pathogenic germs. An important factor which serves to increase the severity of the test conditions and which lies in the susceptibility of the seed to disease, was not taken into allowance when the seed was selected for these tests. For this reason, it was obvious from the very outset that the differences in the efficacy of both preparations would not be so great as when very susceptible seed batches are used. Table 10 gives several final results obtained under conditions similar to those specified for the beet and pea tests.

Table 10: Different types of seed in ordinary compost.

Seed	Rate of application expressed in g of dressing per kg of seed	No. of healthy plants emerged from 100 seeds 3 to 4 weeks after sowing		
		untreated	2.2% Hg as PMA	10% COBH
Flax	2	72	87	91.5
"	2	40	52	71.5
"	2	35.5	54.5	66.8
"	2	70	80	89
"	2	64	82	78
"	2	48.5	64.5	69
"	2	70	88.5	80.5
Cotton with fibres	6	30	37	55
" " "	6	74	84	88
" freed of fibres	6	52	76	76
" " "	6	30	46	49.5
" with fibres	6	26	62	58
" " "	6	20	44	56
" " "	6	62	64	72
" freed of fibres	6	46	56	60
" " "	6	50	54	72
" with fibres	6	64	70	66
Maize	2	22	42	46
"	2	12	62	60
Mustard	2	52	70	76
Cucumbers	2	19	35	59
Tomatoes	2	46	76	74
Ornamental lupins	2	22	28	50

The results show that COBH is able to afford the most different types of seed complete protection provided they are exposed to the influence of pathogenic fungi which are covered by the effective range of COBH.

Soil treatment

The surprisingly high degree of efficacy of COBH applied as a seed dressing against certain phytopathogenic soil fungi implied that the preparation had good prospects of producing excellent results when applied as a soil disinfectant. Therefore, several tests were conducted under normal greenhouse conditions with ordinary compost, sterilized and non-sterilized, using pea seeds, dressed and untreated, with corresponding soil treatment. The powdery active substance was evenly mixed with the soil. Rates of application of less than 20 kg per hectare (2 g per sq. metre) did not produce any real effect. The treated layer of soil was 10 cm deep, so that 2 g per sq. metre correspond to 20 g per cu. metre of soil. Table 11 A gives the results of one test series. A rate of 10 g of COBH per sq. metre produces a good effect, but it is not equal either to that of the same quantity of Hg as PMA or that produced by the COBH seed treatment. Two weeks later, the plants were removed and peas were sown in the same boxes to test the residual effect of the preparations in the soil. The results given in Table 11 B show that COBH has a residual effect in the soil but it is considerably weaker than the effect produced immediately following the soil treatment.

As COBH *in vitro* has no effect against fungi away from the plant, it is improbable that the preparation acts as a disinfectant when applied in the soil.

Table 11: Marrow peas, Alderman E 53.

Test No.	Germinating medium compost	Seed treatment	Soil treatment	A No. of healthy plants emerged from 100 seeds with soil treatment immediately before sowing	B 14 days before sowing
1	sterilized	none	none	76	88
2	"	2.2 % Hg as PMA 2 g/kg	"	80	83
3	"	10 % COBH 2 g/kg	"	86	85
4	not sterilized	none	"	12	3
5	" "	2.2 % Hg as PMA 2 g/kg	"	48	29
6	" "	10 % COBH 2 g/kg	"	80	57
7	" "	none	PMA 100 kg/ha	36	3
8	" "	none	PMA 20 kg/ha	87	65
9	" "	none	COBH 20 kg/ha	24	13
10	" "	none	COBH 100 kg/ha	58	26

It would be more reasonable to assume that it is absorbed by the roots and acts inside the plant. Consequently the residual effect lasting fourteen days must be attributed to a corresponding residue of COBH in the soil, and not to a disinfecting effect, with a subsequent incomplete regeneration of the soil flora. This interpretation of the result is confirmed by the following sowing test which was conducted in an air-conditioned greenhouse at a constant air temperature of 15 °C without direct solar radiation in winter daylight. Untreated peas were sown in ordinary, infested compost treated with COBH at the rate of 25 g/m³ and 100 g/m³, and by way of comparison untreated peas and peas dressed with PMA and COBH were sown in untreated compost. Further quantities of compost mixed with 50 and 200 g of COBH/m³ were prepared ready for a second sowing after 4 days, a third sowing after 10 days and a fourth sowing after 20 days. Immediately before each sowing operation, the treated compost was extended in a ratio of 1:1 with untreated compost which had also been stored under the same conditions in an air-conditioned greenhouse. Table 12 gives the number of healthy plants which emerged from 100 seeds, and which were counted in each test three weeks after sowing. The decrease in the effect of the COBH soil treatment after a period of time, gives definite proof of a loss of active substance in the soil.

The special effective potency demonstrated by COBH when applied as a seed dressing, is lacking in the soil treatment. From this it may be concluded that COBH does not act as a disinfectant in the soil, that the protection of the seedling depends to a greater extent upon the concentration of active substance on the seed than upon the quantity of active substance in the soil, and that probably an intraplant effect is produced.

Table 12: Sowing tests using marrow peas, "Wunder von Kelvedon" variety, with soil treatment.

Test No.	Soil treatment	Seed treatment	No. of healthy plants emerged from 100 seeds 3 weeks after sowing			
			1st sowing	2nd sowing	3rd sowing	4th sowing
1	none	none	3	1	6	1
2	"	2.2 % Hg as PMA 2 g/kg	4	5	15	12
3	"	10 % COBH 2 g/kg	40	42	49	50
4	COBH 25 g/m ³	none	18	11	10	5
5	COBH 100 g/m ³	"	31	28	17	4

Mechanism of action of COBH

Conditions which are frequently encountered in the practical cultivation of many different plants, and which can only be mastered by a dressing preparation possessing a very high standard of efficacy, were purposely selected for the tests described on the previous pages. Wherever possible, use was made of diseased and susceptible seed. The soil was heavily infested with a wide variety of pathogens of plant diseases. The light and temperature con-

ditions were relatively unfavourable for plant growth but most suitable for the development of seedling diseases. The results of the numerous tests conducted not only provide valuable data with respect to the practical application of COBH; they also furnish interesting references which help towards clarifying the preparation's mechanism of action. It is most noteworthy that COBH, as a pure organic compound free of heavy metal, and having a high degree of compatibility with plants, should be far more effective than PMA against certain fungal diseases of plants. PMA is one of those organic mercurial compounds with a special effect against seedling diseases, and possessing a high all-round fungicidal potency which can hardly be excelled. From this it may be concluded that here the importance of the power of disinfection for the success of the dressing takes second place to other properties. According to the test results tabulated earlier, it would appear that these properties of COBH lie in the fact that its protective effect is more persistent. Consequently this raises the question of stability, absorption, spread of the active substance in the soil, and the existence of a systemic effect. Moreover, as PMA at the concentrations and rates of application used in the tests, very rapidly and completely eradicates fungal pathogens adhering to the outer surface of the seed in moist soil, it would appear obvious that the subsequent infections against which only COBH is effective, originate from the soil. The bearing which soil infections have upon the varying efficacy of COBH and PMA was clarified by including sterilized soils in the tests.

Ordinary compost was used as the infested seed bed. For the nonpathogenic medium, use was made either of washed-out river sand or of the same compost which was heated twice for two hours to a temperature of 90° C in "Sterilo Type 7 K" supplied by Messrs. Will Teuwen, Constance, leaving an

Table 13: Polygerm sugar beet seed.

Test No.	Type of soil	Healthy emergence points (A) and healthy plants (B) expressed as a percentage of the seeds sown, 4 to 5 weeks after sowing					
		untreated		2.2 % Hg as PMA 6 g/kg		10 % COBH 3 g/kg	
		A	B	A	B	A	B
1 a	Compost, ordinary	32	32	70	118	82	144
b	River sand, washed	64	110	86	146	76	153
2 a	Compost, ordinary	46	62	68	130	79	140
b	River sand, washed	64	110	86	146	76	153
3 a	Compost, ordinary	52	94	68	152	78	176
b	" sterilized	56	124	82	180	60	134
4 a	" ordinary	22	37	43	76	59	126
b	" sterilized	49	114	73	179	69	163
5 a	" ordinary	51	103	63	148	74	172
b	" sterilized	55	130	73	168	62	138
6 a	" ordinary	51	84	42	84	63	116
b	" sterilized	74	159	77	176	83	171
7 a	" ordinary	58	110	67	159	73	159
b	" sterilized	64	148	79	186	72	162

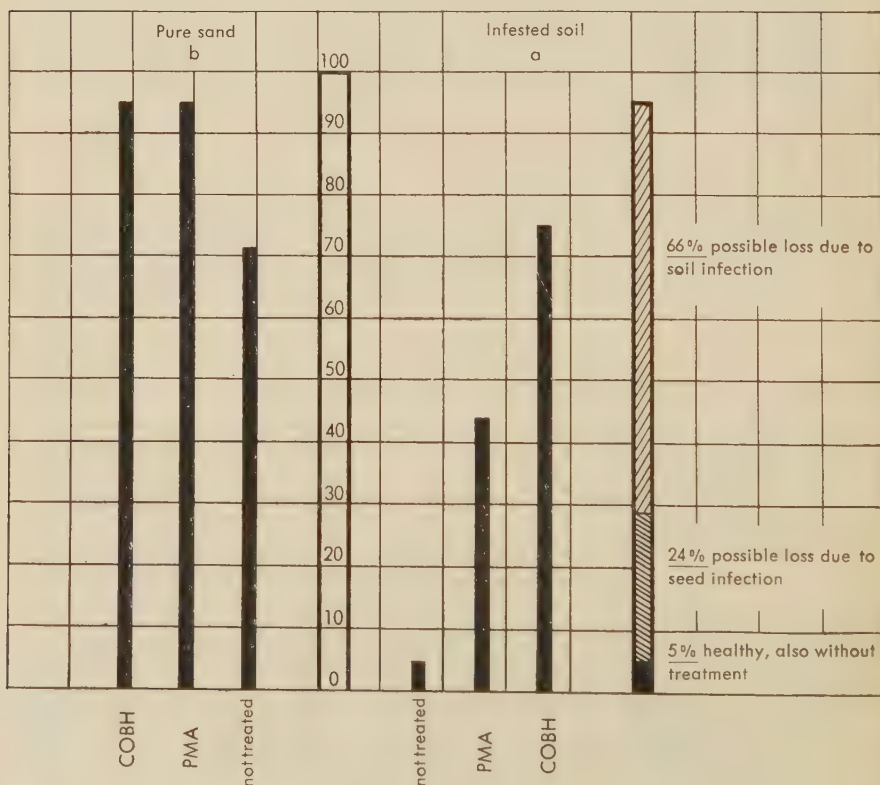
Table 14: Marrow peas, Alderman.

Uniform rate of application: 2 g of dressing per kg of seed.

Test No.	Type of soil	No. of healthy plants emerged from 100 seeds 3 to 4 weeks after sowing		
		untreated	2.2 % Hg as PMA	10 % COBH
1 a	Compost, ordinary	4	37	73
b	„ sterilized	67	88	82
2 a	„ ordinary	10	43	56
b	„ sterilized	83	89	87
3 a	„ ordinary	39	55	84
b	„ sterilized	81	87	85
4 a	„ ordinary	10	46	82
b	„ sterilized	86	86	92
5 a	„ ordinary	5	44	75
b	River sand, washed	71	95	95

Dressing test (No. 5, Table 14)

Peas



interval of 48 hours between each heating process. The comparison tests were all conducted simultaneously using the same seed and under completely similar conditions. The arrangement of the tests and the method of evaluation were exactly the same as before.

The results of the beet and pea tests are completely similar. In infested compost both dressings produce their typical protective effect against seedling diseases the pathogens of which adhere to the seed in certain cases, and are contained in the compost in others. By using sterilized compost or pure sand, the possibility of a soil infection was eliminated in the parallel tests (b), so that only the seed infection could occur revealing the effect of both dressings upon it. In the majority of the tests, the occurrence of seed infection is reflected in the small number of healthy plants which emerged from untreated seed. Both dressings are capable of considerably eliminating disease infestation although here there is practically no difference in their degree of efficacy. Compared with PMA, however, COBH is distinguished by its superior efficacy against soil infections.

The results of the pea test No. 5, Table 14, are interpreted below in greater detail and have been illustrated by means of the graph to provide an example of the quantitative effects. In pure sand, 95 healthy plants emerged from 100 treated peas. The untreated seed produced 71 healthy plants. This meant that the pathogenic germs on untreated seed had caused a loss of 24 plants, which could be avoided by dressing. Moreover, as five of the plants which emerged from 100 untreated peas in infected compost, also remained healthy without chemical protection, it was expected that $5 + 24 = 29$ plants would remain healthy as the result of a seed surface disinfection in the same soil. The PMA dressing, however, gave 44 healthy plants, and 75 were obtained following seed treatment with COBH. Consequently PMA and COBH protected 15 and 46 seedlings respectively against soil-borne infection. With an equal degree of effectiveness against seed-borne seedling rot, COBH produced here an effect against soil-borne infections which was three times greater than that of PMA. It is assumed that during the swelling and germinating processes, COBH penetrates into the seedlings where it gives protection against infection, and also continues to be effective after the seedling has already grown to a stage where it is outside the effective zone of the dressing. PMA, on the other hand, apparently affords the seedling protection against soil-borne infections only as long as the seedling is still within the effective zone of the dressing, which anyway does not extend very far around the seed. The absorbability of COBH by the plant is also supported by the fact that the xylem turns brown to a height of several centimetres when young pea plants are dipped by their undamaged roots in a weakly alkaline solution of COBH. The concentration of the solution was adjusted to 0.01 % so that no injuries were perceptible on the plants. The pea test (Table 15) described below gives further details on this point.

"Alderman" marrow peas were used for the test. Some were left untreated (1), and others were treated dry in the normal manner with 2.2 % Hg as PMA (2), 10 % COBH (3) and 100 % COBH (4) at a rate of 2 g per kg of seed. 2 batches of 50 seeds per test series were sown in wooden boxes, some of which were filled with ordinary, infested compost and others with the same soil but which had been sterilized twice in "Sterilo" at an interval of 48 hours, and at a temperature of 90 °C. The boxes were kept in the unheated greenhouse at summer temperatures of approximately 20 °C.

Batches of the peas which in the meantime had swelled or germinated, were removed carefully from their seed bed, without being injured, at intervals of 1, 3 and 5 days after sowing, placed on a sieve under the sprinkler, and thoroughly washed with water. This treatment removed all soil adhering to the seed and completely eliminated the distance effect of the dressing. Moreover, most of the active substance still present on the seed was also washed off. Then the swollen seeds and young seedlings were planted out in fresh soil. In test series A, the seeds and seedlings were transplanted from sterilized soil back to fresh sterilized soil, in test series B from sterilized soil to ordinary compost, in series C from ordinary compost to sterilized soil, and in test series D from ordinary compost back to ordinary compost. Test series A and D were conducted as checks, and are intended to characterize the possible extreme cases for the seed treatments 1 to 4 and the transplantations 1 to 3

Table 15:

Soil and dressing preparation	No. of healthy plants which emerged from 100 planted peas 18 days after sowing		
	I	II	III
	washed 1 day	and transplanted 3 days	after 5 days
A Sown in sterilized soil; trans- planted in sterilized soil			
1 Untreated	94	94	87
2 2.2 % Hg as PMA	85	87	87
3 10 % COBH	87	92	82
4 100 % COBH	88	93	94
B Sown in sterilized soil; trans- planted in infested soil			
1 Untreated	8	66	75
2 2.2 % Hg as PMA	37	72	85
3 10 % COBH	62	87	95
4 100 % COBH	83	88	88
C Sown in infested soil; trans- planted in sterilized soil			
1 Untreated	55	12	20
2 2.2 % Hg as PMA	82	44	68
3 10 % COBH	94	76	89
4 100 % COBH	84	89	92
D Sown in infested soil; trans- planted in infested soil			
1 Untreated	10	22	14
2 2.2 % Hg as PMA	38	49	71
3 10 % COBH	66	89	86
4 100 % COBH	79	84	92

with and without sources of infection in the soil. Table 15 gives the numbers of healthy plants which emerged from each batch of 100 seeds after a test period of 18 days.

There was no really perceptible outbreak of disease in sterilized soil (test series A). Consequently there was no seed-borne infection, either. This meant that the dressing could not exercise its effect. 100 sown peas, not influenced by dressing preparation, washing or transplantation, produced an average of 90 healthy plants. In infested soil (test series D), the effect of the dressing preparation is in the end most pronounced following transplantation III, and here it is most surprising to note that the efficacy of PMA is almost equal to that of COBH. The figures given in section D I and D II of Table 15 show that the washing and transplantation of the swollen and germinated seeds at intervals of 1 and 3 days after sowing, considerably weakened the effect of PMA whereas the elimination of the COBH distance effect had hardly any influence upon the protective effect. Therefore, it can only be assumed that COBH, in contrast to PMA, is absorbed by the seedlings when the seeds swell and germinate, so that it gives protection inside the plant for several days.

In test series B the beginning of the possible infection is timed exactly while in test series C a precise determination is made of the time when the possible infection comes to an end. The transplantations are most perceptible due to the change of seed bed. The untreated seed produced on an average throughout the entire test, 14 healthy plants from 100 seeds in infested soil, that is when the pathogenic germs in the soil were allowed to act for at least five days on the seeds and seedlings. When the average maximum for the emergence of healthy plants amounts to 90 out of 100 seeds, then a loss of 76 plants must be attributed to soil-borne infections. Assuming that the possible soil-borne infection is equal to 100, the loss in test series BI/1 will be 100 %. This means that an unrestricted soil-borne infection is still possible 24 hours after sowing. In test series BII/1, the loss amounts to only 30 %, which implies that the seedlings have to a considerable extent passed out of the susceptibility stage three days after the seeds are sown. In test series BIII/1, the loss is down to 20 %, and although the seedlings have not yet acquired full powers of resistance, these powers have nevertheless been strengthened again. Test series CI/1 shows that the infection might become manifest to 50 % during the first 24 hours after the seeds are sown. It is evident from CII/1 and CIII/1 that a maximum soil-borne infection can occur during the first three days after sowing. If this infection is prevented in some form or other during this period, the susceptibility stage of the seedlings, which under the above-mentioned test conditions continues for at least five days, is definitely long enough to permit a partial infestation to occur. In test series BI/2, PMA only has a weak action 24 hours after the distance effect of the dressing has been eliminated. This action must be attributed to a small residue of active substance on the peas. If the distance effect of PMA, eliminated due to transplantation to infested soil, does not disappear until after 3 or 5 days (BII/2 and BIII/2), there is no further occurrence of serious infestation. This phenomenon, however, as tests BII and BIII/1 show, must be attributed to an increase in the resistance of the seedlings as they grow older, and does not furnish any information on the persistence of PMA. The effect of COBH, however (BI—III/3 and 4) is not considerably influenced by the early elimination of the distance effect of the

dressings, which only serves to strengthen the theory that COBH produces an intraplant effect. In test series CI/2, PMA is fully effective during the first 24 hours, while the seeds are swelling. However, if the PMA-treated seed remains exposed to infection for 3 to 5 days in contaminated soil, the residual effect of the preparation will prove to be inadequate. COBH, on the other hand, was fully effective in test series C not only during the first 24 hours but also during the whole period that a possibility of infection existed.

In view of these results, we arrive at the conclusion that COBH owes its better effect against soil-borne infections to the fact that its protective action is not limited by the stability and spatial expansion of the dressing's distance effect, but that it is able to penetrate into the seedling where it is translocated, and to remain endoprophylactically effective for several days. However, it has not yet been clarified how the endoprophylactic and restricted systemic effect is produced. Since the substance is not particularly effective *in vitro* but is very effective *in vivo*, it may be assumed that it is converted into an effective compound in the plant or, as an alternative possibility, it makes the plant resistant by some means or other.

The protective effect is the main feature of the COBH action, although it was possible to prove the existence of a slight but positive curative effect. Untreated peas were placed for 2 and 4 days in infested compost to swell and germinate, and exposed to the soil-borne infection. After being washed thoroughly and transplanted in pure sand, only a few healthy plants developed from 100 seeds. However, when the germinated seeds were thoroughly wetted with a 0.005 % COBH suspension after they had been transplanted, it was possible to prevent rot occurring on the seedlings of some of the infected seeds, and a considerably higher percentage of healthy plants was obtained.

Soil-borne infections

We chiefly used, and with good results, ordinary compost of heterogeneous composition with complete and incomplete rotting, obtained from several market gardens, as the general source of infection for soil-borne seedling diseases. The different types of compost supplied to us only varied slightly in their pathogenicity. With a slow emergence of susceptible seeds being the result of the given temperature conditions, all the batches of compost caused a high loss of plants due to seedling rot, and consequently made it possible to carry out a thorough testing of the dressings in a seed bed which from the mycological aspect was most versatile. In our opinion, sowing tests in ordinary compost constitute the best method for obtaining results which will permit a general and reliable opinion to be passed on dressings with respect to their effectiveness against seedling diseases. We also used compost for sowing tests with defined infestation of the soil, in order to provide as far as possible all species of fungus included in the test with suitable organic soil components in the form of the same heterogeneous substrate. Before it was inoculated with the different fungi, the compost was sterilized thermally in the manner already described earlier.

"Fruhstorfer" standard soil, which is sold as a fungus-free soil mixture, reacted as expected like washed-out sand or sterilized compost, with respect to pathogenicity. Soil-borne infections were not established.

Natural outdoor soils, when mixed, would be expected to differ to a greater extent than various types of compost which originate from extremely heterogeneous plant refuse. This assumption was confirmed experimentally. Table 16 gives the results of several typical tests which were carried out with different soils in direct comparison with ordinary compost. The soil from an orchard with perennial turf, produced the best stand irrespective of the seed dressing, while the soil from the beet field caused serious losses which could be reduced by dressing the seed, and in fact be practically eliminated in the case of peas. The garden soil produced average results. Both the garden soil and the soil which was constantly covered with turf in the orchard, are of the same soil type from the Rhine plain near Leverkusen. This soil is a weakly humous, loamy sand.

Table 16:

Test No.	Type of soil	Healthy emergence points (A) and healthy plants (B) expressed as % of the beet seed and peas sown, determined 4 to 5 weeks after sowing					
		untreated		2.2 % Hg as PMA		10 % COBH	
A) Polygerm sugar beet seed				6 g/kg		3 g/kg	
		A	B	A	B	A	B
1 a	Compost	32	32	70	118	82	144
1 b	Garden soil	40	48	76	124	64	110
2 a	Compost	44	78	44	90	68	128
2 b	Soil from own orchard	80	188	76	184	82	180
3 a	Compost	23	37	66	145	75	179
3 b	Field soil						
	Previous crop: Beets	8	8	18	23	41	77
B) Marrow peas, "Alderman"				2 g/kg		2 g/kg	
4 a	Compost		4		34		81
4 b	Field soil						
	Previous crop: Beets		3		72		87

Therefore, the differences in pathogenicity may be attributed chiefly to the different culture state of the soils.

Several check tests, in which the reaction of a soil was adjusted from pH 6·8 to pH 5·7 and pH 7·3, showed that the pathogenicity of the soil is influenced by the actual acidity, but not the efficacy of the dressing used.

The temperature prevailing in the soil after the seed has been sown, also has a great bearing upon the occurrence of seedling diseases. We were also interested in establishing whether brief temperature influences exerted before the seed is sown, can affect the biological balance of the soil to such an extent that a change is also brought about in the pathogenicity of the soil. In tests carried out to investigate this problem, compost was subjected to temperatures of 0, 10 and 20 °C for a period of fourteen days before the seed was sown. In contrast to the temperatures prevailing after sowing, they did not appear

to have any influence upon the later infection of seedlings of beets, peas, beans, maize and flax.

The testing of the dressings against isolated soil fungi which cause seedling diseases, was also carried out by means of sowing tests conducted in the manner already described earlier; compost, which was sterilized by heat application and then inoculated, was used as the seed bed. The soil was inoculated by three different methods.

Method I: The different species of fungi were developed on 20 ccs of suitable agar nutrient media in 200 ccs wide-necked Erlenmeyer flasks. After the bases had been completely overgrown, the flasks were filled up under sterilized conditions with a sterilized mixture consisting of three parts by volume of moist, powdered peat, one part by volume of sand, and one part by volume of seed powder. In order to enable the fungi to become adapted very quickly to the future host plants, the same seed in crushed form was added to the peat-sand mixture, that was used later for the germination tests. As soon as this substrate had become completely overgrown with mycelium, 100 ccs of it were taken as the infector material and mixed evenly with 4 litres of sterilized compost, which was then used in this form for the sowing tests.

Method II: 60 ccs of a thick fungus overgrowth on thin agar substrate were disintegrated and mixed for 10 seconds with 200 ccs of moist.

Table 17: Polygerm sugar beet seed in inoculated and non-inoculated, sterilized compost.

Inoculation of sterilized compost used as germinating medium	No. of healthy emergence points (A) and healthy plants (B) obtained from 100 beet seeds					
			2.2 % Hg as PMA		10 % COBH	
	untreated		6 g/kg		3 g/kg	
	A	B	A	B	A	B
Counts made 2 weeks after sowing						
1 a not inoculated						
1 b <i>Pythium debaryanum</i> (Method I)	84	156	82	192	84	220
	20	20	30	30	50	76
2 a not inoculated	82	138	80	186	72	160
2 b <i>Pythium debaryanum</i> (Method III)	6	6	8	10	46	62
2 c <i>Pythium ultimum</i> (Method II)	32	46	46	74	62	102
2 d <i>Pythium ultimum</i> (Method III)	0	0	0	0	20	32
Counts made 5 weeks after sowing						
3 a not inoculated	61	124	80	177	70	149
3 b <i>Pythium debaryanum</i> (Method II)	33	54	36	59	60	92
3 c <i>Pythium debaryanum</i> (Method III)	1	1	0	0	34	49

sterilized, powdered peat in a "Starmix" type mixing machine. This mixture was spread on sterilized compost in sterilized clay dishes, and used as a seed bed. Afterwards the seeds were covered with a 3 cm thick layer of sterilized compost. This method incurred less work than the other two but often it only produced unsatisfactory infections.

Method III: Disks the size of a pfennig were punched out of fungus cultures in Petri dishes by means of a cork borer. These disks were then laid under each seed when they were sown out. The infection proved to be most successful. The germination dishes, each inoculated with a different fungus, were kept so far apart that it was impossible for fungi to be transmitted from one dish to the other by splashes of water made during the "watering" process.

In the germination tests conducted with peas and sugar beets, our strains of *Alternaria tenuis* Nees, *Botrytis cinerea* Persoon, *Phymatotrichum omnivorum* (Shear) Duggar, and *Rhizoctonia solani* Kühn were not pathogenic. *Pythium debaryanum* Hesse and *Pythium ultimum* Trow, on the other hand, proved to be extremely virulent to beets and peas, so that it was possible to give the dressings a thorough testing with these species.

The results of several tests are given in Tables 17 and 18. They clearly illustrate the effect of COBH applied as a seed dressing against soil-borne seedling infections caused by *Pythium debaryanum* and *Pythium ultimum*, thus confirming in principle the experience gained with naturally infected soils.

Table 18: Marrow peas, "Alderman" variety, in inoculated and non-inoculated, sterilized compost.

Inoculation of sterilized compost used as germinating medium	No. of healthy plants emerged from 100 seeds 4 to 5 weeks after sowing		
	untreated	2.2 % Hg as PMA 2 g/kg	10 % COBH 2 g/kg
1 a not inoculated	78	76	82
1 b <i>Pythium debaryanum</i> (Method I)	14	10	74
2 a not inoculated	82	94	94
2 b <i>Pythium debaryanum</i> (Method III)	28	70	86
2 c <i>Pythium ultimum</i> (Method III)	0	0	70
3 a not inoculated	79	82	77
3 b <i>Pythium ultimum</i> (Method III)	13	9	60

Discussion of the results

The control of seedling diseases is unsatisfactory, firstly because the pathogens not only occur on the seed but also exist in the soil, and secondly because the organic mercurial compounds generally used as seed dressings merely give the seed surface disinfection without affording the seedlings protection against

soil-borne infections. The search for better preparations and their approval as being suitable for practical application, is complicated due to methodical difficulties encountered in the biological test. During the past five years, we have attempted to develop a dressing which is also effective against soil-borne infections. In this paper, we have given a summarized report on the discovered preparation, quinone oxime benzoyl hydrazone (COBH), with details of the experience gained from the methodical aspect in the biological test.

Material and Method

It has been illustrated that tests conducted on dressings *in vitro* may present a completely misleading picture. It is commendable to carry out tests under conditions which are more or less natural but which can be controlled. Such conditions can be produced in greenhouses or in air-conditioned vegetation chambers. Outdoors, the success of such tests depends too much upon chance, i. e. upon factors which can neither be pre-determined nor influenced. Far too often, field tests cannot be evaluated because seedlings diseases fail to occur even on untreated seed, or else other losses are caused which have no connection with seedling infections originating from fungi. In order to assess the effectiveness of dressings against damping-off, it has proved advisable to establish the number of healthy plants produced from a given quantity of seed. Very often, an opinion is passed on a dressing based on the state of health of the emerged plants, without making any allowance for the quantity of seed used, either according to the numbers or at least the weight as the standard unit. This procedure must automatically lead to wrong conclusions because mostly the infected seedlings perish even before they reach the surface of the soil. Even determinations of yields are unsuitable as a basis for assessing the value of dressings because there are too many other factors which have a bearing upon the yield. The number of healthy plants must be counted at a time when no subsequent infections are to be expected. Normally, the plants have outgrown the susceptible seedling stage about fourteen days after the seed has been sown so that by making allowance for the period of incubation, it should be possible to carry out the final evaluation three to five weeks after sowing. The different seed varieties, and in fact seed of different origin and from different batches, vary considerably with respect to the susceptibility of their seedlings to seedling diseases. Therefore, in order to give the dressings a severe testing, it is advisable to select suitable seed by conducting preliminary tests. The susceptibility of the seedlings appears to have a greater bearing upon the occurrence of seedling diseases, than the nature and severity of seed infection. Furthermore, special importance is attached to the selection of the seed bed as the source of infection when carrying out a test on seed dressings, which should not be restricted solely to determining to what extent there is a disinfection effect on the surface of the seed.

For this purpose, ordinary compost should be given preference over all other natural and artificial soils because it constitutes a very general substrate. Under favourable conditions, this germinating medium offers the best guarantee for a serious occurrence of seedling diseases. It contains a fairly natural, and in most cases also a widely varied mixture of soil organisms which in many ways are interdependent upon each other. Provided the compost is of heterogeneous composition, the location of its origin is of secondary importance.

As a rule, natural soils are less versatile, and therefore they differ greatly in their pathogenicity dependent upon their origin, so that results obtained only apply to the particular case in question. Standardized soils can to a large extent be defined and reproduced exactly. They can prove to be irreplaceable for special problems, particularly with respect to the influence of abiotic factors of the soil. On the other hand, they appear to be too one-sided as a substrate for a natural soil flora, that is for use in the general testing of dressings against damping-off. We also gave preference to compost for sowing tests with defined artificial infection of the soil, so that by using the same heterogeneous substrate suitable organic soil components would be available as far as possible to all the species of fungi included in the tests. The compost was sterilized by means of heat application before the inoculation.

In addition to selecting a susceptible and infected seed, and using infested compost, there are other factors to be considered in order to obtain a serious infection. In particular, the soil temperature greatly influences germination and growth. At relatively low temperatures, the development of the seedlings is delayed, their powers of resistance are weakened, and the susceptible stage is prolonged. However, the soil temperatures should not be so low that pathogenicity of the fungus is also affected. In order to obtain maximum infestation, a study must be made of the most favourable temperature ranges for the different parasite-host combinations. In our experiments the optimum was chiefly between 10 and 15°C. During the cold season, these temperatures are relatively easy to keep up in the greenhouse. In warmer weather, it is mostly essential to place the seed boxes in a cool place for about 10 days after the seed has been sown. With direct solar radiation, the temperature of soil layers near the surface often varies considerably from the air temperature. Furthermore, a certain degree of etiolation assists the occurrence of infestation so that weak light on short days may contribute most noticeably to making the test conditions more severe. As far as possible, the degree of moisture was kept even throughout the soil with the humidity at normal. In this respect, no experimental variations were made.

Dressing effect

In this biological test, COBH was always in direct comparison with phenyl mercury acetate (PMA). PMA is one of the conventional types of organic mercurial compounds which do not differ in their mode of action, and which have a high all-round fungicidal potency. PMA is also one of the best, particularly with respect to its effect against seedling diseases. In view of the results obtained in comparison tests, the knowledge gained with PMA can quite safely be applied in principle to other conventional mercurial compounds.

In vitro, COBH did not produce a really perceptible fungicidal or fungistatic effect against any of the test fungi used. It is not until it is used in combination with swelling and germinating seeds that a strong effect is produced against seedling diseases caused by fungi. In sowing tests where soil-borne infection was eliminated by using pure sand or sterilized soil, the effect of COBH was equal but not superior to that of PMA against seed-borne infections of different seeds. In naturally or artificially infested soils, however, COBH gives evidence of its superior efficacy when applied as a seed dressing.

In an artificially inoculated seed bed, definite proof was furnished of the preparation's effect against the soil fungi, *Pythium debaryanum* and *Pythium ultimum*. On the basis of experience gained with naturally infected soil, an effect against *Aphanomyces laevis* is very likely but improbable against *Rhizoctonia solani*. COBH is fully effective at the same low concentrations and rates of application normally used for organic mercurial compounds. Large overdoses are tolerated without any harm whatsoever being caused, which means that the chemotherapeutic index is extremely favourable.

It was proved for organic mercurial compounds that apart from the surface disinfection of the seed, their effect does not extend in the soil more than 2 mm away from the seed. Within this effective zone of the dressing, certain protection is also afforded against soil-borne infections but only as long as the seedling is still in this zone. As soon as the seedling becomes bigger, it is completely unprotected, being outside the dressing's effective zone. In contrast, COBH affords protection against infection to all parts of the seedling for a period of 10 to 14 days. By the end of this time, the seedling has, as a rule, already grown out of the susceptible stage. It was thought that the reason for the prolonged protective effect which seemed to extend over a greater distance, might be due to the preparation having a greater distance effect as a dressing. But in view of the slight solubility of the preparation in water, and its extremely low vapour pressure, it is improbable that it spreads better in the soil than PMA. It was also proved that the protective effect of COBH remains persistent when the treated seed and the emerged seedlings are freed of the dressing adhering to the outside of the seed by being given a thorough washing several days after sowing, a treatment which also destroys the distance effect of the dressing. This fact leads to the conclusion that the preparation has an intraplant effect which spreads systemically. This conclusion is in turn strengthened by the fact that when young plants are immersed by their uninjured roots in a COBH solution, they develop a brown colour corresponding to that of the preparation after a short time. This colour is found chiefly in the central cylinder of the immersed root and in the walls of the xylem of the stem to a height of several centimetres above the level of the liquid.

COBH also produces a certain curative effect which, however, is slight compared with the protective effect brought about by the seed treatment. Proof was given of this curative effect by causing seed to germinate in infected soil, washing it, then transplanting it in clean soil, and finally treating it with COBH solution by means of the soil watering technique with the result that the outbreak of seedling rot was prevented on some of the infected seedlings.

The uniform distribution of COBH in the soil at the rate of 10 g per sq. metre or 100 g per cu. metre also renders it possible to eliminate soil-borne seedling diseases to a large extent. It is presumed that this is not a case of a genuine soil disinfection but again an intraplant effect made possible by the absorption of the preparation from the soil. This method of applying COBH, however, appears to attach no practical significance because the large quantities of preparation it involves are neither justified by a more complete effect nor by a more persistent effect compared with its application as a dressing.

Seed dressing with organic mercurial compounds enables the surface of the seed to be disinfected, consequently eliminating seed-borne seedling diseases to a large extent. By using COBH, it is also possible today to control effectively soil-borne infections in the same manner. The success of the

dressing treatment can be reduced occasionally only when pathogenic soil fungi occur which are not covered by the range of action of COBH, or when the seedlings remain in the susceptible stage for several weeks due to a long period of unfavourable weather with the possibility of an infection occurring after the residual effect of COBH has already disappeared.

Summary

A description is given of quinone oxime benzoyl hydrazone (COBH), a new type of active substance for controlling seedling diseases by means of seed treatment. Its efficacy and mechanism of action are demonstrated by general sowing tests and special experiments, and an endeavour is made to contribute towards clarifying the problem of finding suitable material and methods to test dressings for their effectiveness against seedling diseases.

In view of the fact that seedling diseases are caused both by pathogens on the seed and by pathogens in the soil, it is essential to make allowance when testing the dressings for both sources of infection from the methodical aspect. Ordinary compost is recommended as a general, naturally infected seed bed. The suitability of seed for such investigations is not only determined by the nature and severity of the infection with pathogenic germs but also by the susceptibility of the seed, which may vary considerably dependent upon the origin, variety and batch. All factors which delay the germination and growth of the seedlings, increase and prolong their susceptibility, contribute to making conditions more severe in sowing tests. Relatively low temperatures and poor light are of special importance.

It was established that COBH is practically ineffective *in vitro* but when applied as a seed dressing it is able to eliminate to a large extent not only seed-borne seedling diseases but also soil-borne diseases caused for example by *Pythium debaryanum* and *Pythium ultimum*. This surprisingly good protective effect is not rendered possible by the preparation having a direct influence on the fungi within an extended effective zone of the dressing, but due to the absorption of the active substance by the swelling and germinating seeds, also by its translocation in the seedling. It has not yet been clarified whether the intraplant effect and the systemic protective effect restricted to several centimetres is caused by COBH direct, by a transformation product of the preparation, by the inactivation of toxins, or by the mobilization of resistance factors specific to the host.

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Experience Gained from the Application of a Combined Preparation of 2,4-D and 2,4,5-T Esters for Coppice Conversion

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Publications released to date on the application of 2,4-D and 2,4,5-T esters and their combinations in forestry (cf. references) have not, as far as I know, emphasized the spontaneous and amazing effect of the latter substances on the rapid decomposition and decaying of available stand waste products including young stand caused to perish rapidly by the treatment. This unusual effect appears to stimulate to such an extent the condition of the top soil layers and also the growth and health of the new cultures formed immediately afterwards, that from the forestry aspect it is probably just as important as the other advantages gained from ester applications. — Exact details on this most interesting development will, however, have to be reserved for a later publication. — In this paper it is intended to report at the present only on experience gained from an economic aspect on forest applications of a combination of 2,4-D and 2,4,5-T esters in diesel oil emulsion in order to remove damming-up sprouts of oaks and other broad-leaved trees on coppice conversion areas in the Hessian Forest Supervision District at Chausseehaus near Wiesbaden.

From 1951 onwards, the author carried out a series of trial sprays chiefly with preparations dissolved in water, directed against unwanted flora growing in cutting areas. The excellent results obtained in these trials and the experience confirmed by them that di- and trichlorophenoxyacetic esters are able to give complete eradication even of very rapidly growing sprouts of broad-leaved trees only when the esters are dissolved in diesel oil, led to a large scale test being carried out in June 1954 in the coppice conversion area. 15.5 litres of a commercial type mixed ester made up of one part of 2,4-D and two parts of 2,4,5-T at DM 24 per litre*, dissolved in only 35 litres of diesel oil at DM 0.47, were applied per hectare on an eight-year-old sprout area of maximum density, with an average height of approximately 4 metres. The application was made by means of a portable type motor-powered atomizer, and directed against stem bases and thickenings of the roots up to a height of approximately 50 cm. The atomizer equipped with a 2 HP engine (price of approximately DM 600) treated 2 hectares daily under the given conditions, with an operating crew of two men. The total costs per hectare of treated area amounted to about DM 450.

The result of this large-scale test was referred to by E. Röhrig in „Mitteilungen der Biologischen Bundesanstalt Berlin-Dahlem“, Yearbook 1956, No. 85, Page 203. Although the seriously degenerated coppice thicket contained, in addition to oak, at least twenty other bush and tree species, 90 % of them had died down into the roots within a very short time — with the pleasing exception of several spruces of the same age which apparently were rather

* Editor's note: The Bayer preparation "Tribution" D was used in this test.

resistant. The remaining 10 % are definitely regarded as being desirable as an admixture — perhaps permanent — of broad-leaved trees and as shelter trees and driftwood in the mixed cultures (chiefly larch and Douglas fir) planted during the following Autumn. It is hoped that this remaining 10 % will give at least a temporary undergrowth of broad-leaved trees later. Naturally, varying degrees of effectiveness can be obtained if required by means of uneven atomizing or by interrupting the treatment in strips with a correspondingly lower consumption of emulsion, whereby the costs of the treatment and also those incurred for felling which is necessary later on, can also be varied per hectare. It is, however, not advisable on principle to carry out actual dosaging trials to determine the mixture ratio of active substance and vehicle, and the total consumption of emulsion per unit of area, for the simple reason that this has already been done by the manufacturers.

The economic success of atomizing with 2,4-D and 2,4,5-T ester preparations is to be attributed to the fact that:

1. The killed foliage and woody parts of the treated bush, in comparison with untreated cutting areas, are decomposed at a surprisingly rapid rate and most thoroughly to give excellent mould which evidently is very mild. This provides the best growing conditions for the newly planted cultures — also for herbaceous vegetation but not for grass flora.
2. The phenoxyacetic esters, in contrast to the chlorates, do not cause any delay in the re-planting of the treated area because they are not soil poisons. In fact, from the biological aspect, they are just the opposite of "poisons", being plant growth regulators.
3. The few old broad-leaved tree stumps which still sprout in the next two years cause an expenditure of DM 50 at the most for the 25 working hours spent on levelling the canopy, provided they have to be removed because of the danger of damming-up, and are not left as shelter trees and as an admixture of broad-leaved trees in the newly planted (conifer) cultures.

The decision in favour of or against carrying out a treatment with 2,4-D and 2,4,5-T ester preparations is after all a matter of whether

- a) money is available immediately;
- b) labour is available for a long period.

Because in those cases where it can be arranged for the bush to be removed free of charge while it is still green by gatherers so that no felling costs are incurred, then the costs (approximately DM 450 per hectare) for a treatment with ester preparations will merely represent an advance payment of next year's costs for cutting thus more or less completely eliminated. However, in cases where the green bush can only be removed against payment, then the clearance costs (at least in the afore-mentioned district) practically correspond with the costs incurred for the application of 2,4-D and 2,4,5-T ester preparations. In this instance, therefore, the advance payment does not apply because the course dry bush killed by the treatment with ester preparations can always be got rid of free of charge to gatherers, and the finer bush left over the cultures to protect them against frost and radiation. In both

cases, however, the treatment with ester preparations saves considerable time and labour, although the full benefit of this saving is not felt until a year or two later.

As already mentioned, a total of three days' work per hectare only need be done on the fully treated areas to make later clearances of coppice sprouts and at the most during the following two years, whilst in the untreated areas where the green bush was removed mechanically considerable work will have to be done, probably also during the cultivation year, and for at least three further years, incurring very great expense. Based on present-day wages, a cost of about DM 450 per hectare will always have to be reckoned with, in other words at least the same amount which the treatment with ester preparations also costs. Consequently, from the financial aspect atomizing with ester preparations amounts either only to an advance payment of the costs incurred later for cutting the cultures, or else in those cases where the first purely mechanical clearance of stump sprouts would cost approximately the same as the chemical treatment, it means that these later costs are completely saved.

From the labour aspect, it means a great help at all events because consideration does not have to be given to the time factor. High or even very low temperatures do not influence the effect of ester-oil emulsion. The (dicotyledon) vegetation, however, reacts much more susceptibility and far more rapidly to applications of the emulsion in the first half of the year than in the second. Atomizing should not be carried out, however, during periods of heavy rainfall because the dripping thickets can never be treated with adequate systematic efficiency.

From the silvicultural aspect, this new method of stump sprout clearance guarantees subsequent cultures of all kinds far greater security and enables them to grow more vigorously than the purely mechanical cutting of green coppice stands in which large parts of the subsequent cultures regularly tend to suffocate in new, wildly growing stump sprouts despite all cutting operations.

There is, however, one hazard arising out of the atomizer application of 2,4-D and 2,4,5-T ester preparations, which must be emphatically stressed, namely the drifting of spray clouds when it is windy. These spray clouds may cause serious or fatal harm to neighbouring plots of young broad-leaved trees. Therefore, when it is windy, atomizers should not be used at all, but instead sprayers with a much larger nozzle bore. Treatments should not be carried out at the edges of fields and gardens, or else in cases where applications have to be made in the vicinity of gardens and fields it is advisable to take out a liability policy.

In summarizing, it may be stated that despite the brief experience on the application of 2,4-D and 2,4,5-T ester preparations in forestry, the urgent conversion of the extensive and almost unproductive West German coppices can only be effected by applying chemical preparations particularly as the present-day shortage of labour is becoming an ever greater problem. However, this will provide a prerequisite for a most considerable increase of the output of the total forest acreage in Germany.

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Studies on "Metasystox" Residues, 1956

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To complete the data already published on Page 116 ff. of Höfchen-Briefe No. 2, 1956, regarding toxic residues in different harvest crops following sprays of Metaisosystox applied in 1955*, further studies were conducted this Summer. Whereas the applications were purposely carried out late in 1955 — the sprays were applied from September 26 to 30, in order to obtain the highest possible residual values — we applied the preparation in early Summer this year, in other words on those dates when applications are normally carried out in practice. Once again, a $^{32}\text{phosphorus}$ -labelled preparation was used which was kindly supplied to us by Dr. Schrader of the Plant Protection Laboratory of Farbenfabriken Bayer Aktiengesellschaft, Elberfeld. Just as in the preceding year, we applied Methylisosystox, the main effective component of "Metasystox". The active substance was formulated in conformity with the commercial preparation, and was applied at a concentration of 0.1 % in water based on preparation, at the rates of application recommended for the different crops.

Analytical methods:

- a) To determine the initial values, in other words on day 0 (0 = date of spraying), the plant parts were harvested one hour after the spray coating had dried on, wet-ashed by means of concentrated H_2SO_4 , and then oxidized with concentrated hydrogen peroxide. The preparation was then filled up to give a certain liquid volume and the radioactivity was measured with a liquid counter of 10 ml volume. After allowing for all correction factors, the existing quantity of active substance was then calculated.
- b) All the specimens taken after day 0 were processed according to the method described in Höfchen-Briefe No. 2, 1956*, and the toxic portions of sulphoxide and sulphone were then determined:

10 to 100 g of plant material are minced in the "Starmix" with 5 to 6 times the quantity of methanol, drawn off, and the cellulose residue washed with methanol until it is completely white, and there is no more radioactivity in the filtrate. The methanol distillate is evaporated in vacuum at 40° C water bath temperature, until all the methanol is distilled off. If necessary, it is filled up with water to 30 ml, and shaken out four times with equivalent quantities of petroleum ether. Subsequently it is extracted four times with equal volumes of chloroform. The entire toxic portion is present in the chloroform. The quantity of the toxic portion is determined by measuring the radioactivity, as described above in paragraph a).

From these tests, it is perfectly obvious that following applications of "Metasystox" at the standard rates, treated vegetables and fruit can definitely be used for human and animal consumption 8 to 10 days after the last spray.

* R. Mühlmann und H. Tietz: "The Chemical Behaviour of Methylisosystox in the Living Plant and the Problem of Residues", Höfchen-Briefe 2/56.

Metaisosystox sprayed at 0.1 % on different
cultures from June 14 to July 14, 1956

Culture	No. of sprays	Date of spraying	Date of harvest	Days after spraying	Toxic residue mg/kg = ppm
Vines					
Leaves	1	13. 6.	13. 6.	0	81.0
do.	1	13. 6.	30. 6.	17	3.163
Berries	1	13. 6.	23. 7.	40	0.196
Strawberries					
Leaves	1	14. 6.	14. 6.	0	25.87
Fruit	1	14. 6.	25. 6.	11	0.414
do.	1	14. 6.	10. 7.	26	0.047
Black currants					
Leaves	1	14. 6.	14. 6.	0	32.58
Fruit	1	14. 6.	14. 6.	0	4.85
do.	1	14. 6.	25. 6.	11	0.31
do.	1	14. 6.	10. 7.	26	0.161
Snake melons (in greenhouse)					
Leaves	1	14. 6.	22. 6.	8	1.04
Melons	1	14. 6.	22. 6.	8	0.147
do.	1	14. 6.	4. 7.	21	0.003
Hops					
Leaves	1	14. 6.	14. 6.	0	33.13
do.	1	14. 6.	25. 6.	11	0.224
do.	1	14. 6.	10. 7.	26	0.048
Inflorescences	1	14. 6.	23. 7.	39	0.025
Bush peas, "Wunder von Wephan" variety					
Straw and blooms	1	14. 6.	14. 6.	0	10.06
Straw	1	14. 6.	25. 6.	11	0.978
do.	1	14. 6.	10. 7.	26	0.0298
Pods	1	14. 6.	25. 6.	11	0.8625
do.	1	14. 6.	10. 7.	26	0.0092
Peas	1	14. 6.	25. 6.	11	0.144
do.	1	14. 6.	10. 7.	26	0.0207

Culture	No. of sprays	Date of spraying	Date of harvest	Days after spraying	Toxic residue mg/kg = ppm
Blue-flowering sugar peas					
Straw and blooms	1	14. 6.	14. 6.	0	10.35
do.	1	14. 6.	25. 6.	11	0.23
Straw and young fruit	1	14. 6.	10. 7.	26	0.013
Straw	2	14. 7.	14. 7.	0	10.64
do.	2	14. 7.	23. 7.	9	0.15
do.	2	14. 7.	9. 8.	26	0.012
Pods and peas	2	14. 7.	14. 7.	0	3.0
Pods	2	14. 7.	23. 7.	9	0.284
do.	2	14. 7.	9. 8.	26	0.0051
Peas	2	14. 7.	23. 7.	9	0.033
do.	2	14. 7.	9. 8.	26	0
Early potatoes, sprayed at 0.1 %					
Leaves	1	18. 6.	18. 6.	0	12.23
do.	1	18. 6.	2. 7.	14	0.091
Potato tubers	1	18. 6.	18. 7.	30	0.0028
Early potatoes, sprayed at 0.2 %					
Leaves	1	18. 6.	18. 6.	0	25.86
do.	1	18. 6.	2. 7.	14	0.133
do.	1	18. 6.	18. 7.	30	0.024
Potato tubers	1	18. 6.	18. 7.	30	0.0069